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(54) NEW PHARMACOLOGICAL ACTION OF POLYSULFATED MUCOPOLYSACCHARIDES

(57)Abstract:
PROBLEM TO BE SOLVED: To inhibit hyperplasia of endangium, inflammatory responses and inflamamtory diseases.
SOLUTION: This selectin-dependent cell adhesion inhibitor, an adhesion molecule expression inhibitor of skin keratinized cells, an inhibitor of endangium hyperplasia and angiostenosis or a prophylactic or a therapeutic agent for skin microbisms or the like comprises polysulfated mucopolysaccharides as an active ingredient.

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* NOTICES *

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CLAIMS

[Claim(s)]
[Claim 1] The selectin dependency cell adhesion inhibitor which makes a multi-sulfation mucopolysaccharide an active principle.
[Claim 2] The blood vessel intima hyperplasy and the vasoconstriction inhibitor which make a multi-sulfation mucopolysaccharide an active principle.
[Claim 3] The prevention or the remedy of arteriosclerosis which makes a multi-sulfation mucopolysaccharide an active principle.
[Claim 4] The adhesion-molecules manifestation inhibitor of the skin keratinization cell which makes a multi-sulfation mucopolysaccharide an active principle.
[Claim 5] The prevention or the remedy of the skin microbism which makes a multi-sulfation mucopolysaccharide an active principle.
[Claim 6] The prevention or the remedy of graft versus host disease which makes a multi-sulfation mucopolysaccharide an active principle.
[Claim 7] The prevention or the remedy of ********** which makes a multi-sulfation mucopolysaccharide an active principle.
[Claim 8] The prevention or the remedy of the psoriasis based on the adhesion-molecules manifestation inhibitory action of a skin keratinization cell which makes a multi-sulfation mucopolysaccharide an active principle.

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DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates to a selectin dependency cell adhesion inhibitor, an adhesion-molecules manifestation inhibitor of a skin keratinization cell, etc. which make a multi-sulfation mucopolysaccharide an active principle.
[0002]
[Description of the Prior Art] If the load of the high cholesterol foods is carried out to an ape, the image with which the leucocyte has pasted up and permeated will be accepted in the main artery blood vessel inner bark. Moreover, since the same image is observed also in a youth's example of ****, it is thought that it is important as an initial lesion of adhesion to the blood vessel inner bark of this leucocyte and arteriosclerosis formation of infiltration.
[0003] If inflammation is caused, the leucocyte which circulates in blood will begin (rolling (rolling)) to roll, will paste up soon firmly the vascular endothelial cell top activated by cytokine etc. (firm adhesion), it passes through a blood vessel gap (transmigration), and hyperplasy of the blood vessel intima is carried out, or it puffs up an inflammatory response. It is known that the selectin discovered on a leucocyte or a vascular endothelial cell and sugar chains (selectin ligand), such as serial RUISU X discovered on a leucocyte or a vascular endothelial cell, will participate in this rolling of a series of. It is known that the molecule group called the integrin discovered on a leucocyte and the immunoglobulin super family discovered on an endothelial cell to the next adhesion will involve. Therefore, if these phenomena are blocked and adhesion to the vascular endothelial cell of a leucocyte is checked, controlling the infiltration to the outside of the blood vessel of a leucocyte, and controlling the hyperplasy and the inflammatory response of blood vessel intima is expected.
[0004] Moreover, ICAM-1 (intercellular adhesion molecule -1) which is adhesion molecules is the molecule first identified as ligand of LFA-1 (lymphocyte function associated antigen -1). At the time of inflammation, it pastes up with a vascular endothelial cell through ICAM-1/LFA-1, and a leucocyte permeates an inflammation part. Possibility that the ICAM-1 manifestation of the keratinocyte (KC) especially in the inflammatory diseases (the lichen planus, psoriasis, contact dermatitis, etc.) of epidermis was making the inductive role infiltration on the epidermis of a LFA-1 positivity lymphocyte was reported (1358 skin clinical 35 (8) **: 33;1343- 1993). Therefore, control of an inflammatory disease is expected by checking the ICAM-1 manifestation of KC.
[0005] On the other hand, it is known that the multi-sulfation chondroitin sulfate (heparinoid from animal organs) which is one sort of a multi-sulfation mucopolysaccharide has blood coagulation depressant action, a peripheral blood liquid circulation promotion operation, and fibrocyte growth depressant action. Moreover, multi-sulfation chondroitin sulfate has a skin moisturization operation, and usefulness is known by drying skin disease, such as a xerosis, astaatosis, and keratodermia tylodes palmaris progressiva. However, the arteriosclerosis depressant action by the above-mentioned mechanism, skin bacterial infection inhibitory action, etc. were not known.
[0006]

[Means for Solving the Problem] this invention persons found out having the selectin dependency cell adhesion inhibitory action excellent in multi-sulfation mucopolysaccharides, such as multi-sulfation chondroitin sulfate, the adhesion-molecules manifestation inhibitory action of a skin keratinization cell, etc., as a result of inquiring about the new application paying attention to a multi-sulfation mucopolysaccharide.
[0007] That is, this invention offers the following inhibitors and prevention, or a remedy.
The selectin dependency cell adhesion inhibitor which makes an active principle a term 1. multi-sulfation mucopolysaccharide.
The blood vessel intima hyperplasy and the vasoconstriction inhibitor which make an active principle a term 2. multi-sulfation mucopolysaccharide.
The prevention or the remedy of arteriosclerosis which makes an active principle a term 3. multi-sulfation mucopolysaccharide.
The adhesion-molecules manifestation inhibitor of the skin keratinization cell which makes an active principle a term 4. multi-sulfation mucopolysaccharide.
The prevention or the remedy of the skin microbism which makes an active principle a term 5. multi-sulfation mucopolysaccharide.
The prevention or the remedy of graft versus host disease which makes an active principle a term 6. multi-sulfation mucopolysaccharide.
The prevention or the remedy of ********** which makes an active principle a term 7. multi-sulfation mucopolysaccharide.
The prevention or the remedy of the psoriasis based on the adhesion-molecules manifestation inhibitory action of a skin keratinization cell which makes an active principle a term 8. multi-sulfation mucopolysaccharide.
[0008] Furthermore, this invention offers prevention or the remedy of the blood vessel illness based on the adhesion inhibitory action of the leucocyte and blood vessel inner bark which make a multi-sulfation mucopolysaccharide an active principle. Moreover, this invention offers the prevention or the remedy of the skin illness based on the adhesion-molecules manifestation inhibitory action of a skin keratinization cell which makes a multi-sulfation mucopolysaccharide an active principle.
[0009]
[Embodiment of the Invention] The multi-sulfation mucopolysaccharide used by this invention means what was compounded by introducing a sulfuric-acid radical into the long-chain polysaccharide which has the repeat unit of the disaccharide which consists of hexosamine, uronic acid, or a galactose chemically. Since a thing with a sulfuric-acid radical also exists in the mucopolysaccharide of the natural origin, what many sulfurated them still more chemically is included by the multi-sulfation mucopolysaccharide of this invention.
[0010] Moreover, the multi-sulfation mucopolysaccharide of this invention can also be used if needed as a salt gestalt which is acquired by the salt formation reaction using the hydroxide of alkali metal, such as sodium and a potassium, a carbonate, or amines and which is permitted physiologically.
[0011] In this invention, although hexosamine is large and the hydroxyl of a hexose means the compound permuted by the amino group, specifically, D-glucosamine, D-galactosamine, etc. are mentioned.
[0012] Moreover, although uronic acid means widely what the primary alcohol of an aldose oxidized and became a carboxyl group, the uronic acid of the natural origin, such as D-glucuronic acid, L-iduronic acid, D-galacturonic acid, D-mannuronic acid, and L guluronic acid, is specifically illustrated.
[0013] As an example of a mucopolysaccharide, chondroitin sulfate (a chondroitin 4-sulfuric acid, chondroitin 6-sulfuric acid, etc.), a heparan sulfate, a keratan sulfate, dermatan sulfate, hyaluronic acid, chondroitin, etc. are mentioned.
[0014] moreover, as a multi-sulfation mucopolysaccharide, multi-sulfation chondroitin sulfate, a multi-sulfation heparan sulfate, a multi-sulfation keratan sulfate, multi-sulfation dermatan sulfate, multi-sulfation hyaluronic acid, etc. mention — having — multi-sulfation chondroitin sulfate, such as heparinoid from animal organs (henceforth MPS) given in Japanese

Pharmaceutical Codex, chondroitin sulfate D, and chondroitin sulfate E, and a mole cricket — a tongue poly sulfuric acid is desirable. It is multi-sulfation chondroitin sulfate, such as MPS, still more preferably.
[0015] Although especially the multi-sulfation mucopolysaccharide of this invention does not limit the origin, the thing which it comes to sulfurate by performing sulfation processing artificially, for example to the above-mentioned mucopolysaccharide as a more suitable thing is mentioned.
[0016] Moreover, although especially the number of the sulfuric-acid radicals which the multi-sulfation mucopolysaccharide of this invention has is not limited, it per monosaccharide and usually [an average of 0.55-5] has it at a rate of an average of 0.7-2 pieces more preferably an average of 0.6-2.9 pieces.
[0017] Although the molecular weight of the multi-sulfation mucopolysaccharide used by this invention or its salt differs and is not limited by the class of polysaccharide, either, it is [5000 to about 1 million / 10000 to about 100000] more preferably desirable [molecular weight] that it is 10000-50000 further much more preferably preferably [the average molecular weight is 1000 to about 10 million in number average molecular weight, and].
[0018] As an approach of introducing a sulfuric-acid radical into a mucopolysaccharide, a known approach, for example, a mucopolysaccharide and a sulfating agent, is warmed in a suitable solvent, and the approach of making it react is mentioned. Although it is not limited especially if the purpose of many sulfation can be attained as a sulfating agent, it is desirable to use complexes, such as a sulfuric anhydride, a pyridine, or triethylamine. Although the operating rate of a mucopolysaccharide and a sulfating agent can be chosen as arbitration according to the desired sulfation percentage (or sulfur content) and desired reaction condition of a multi-sulfation mucopolysaccharide, generally it is used at a rate which serves as 2 - 10 weight section to the mucopolysaccharide 1 weight section. As a solvent, protophilic solvents, such as dimethylformamide, can be mentioned, for example. Although it is not limited as reaction temperature and reaction time especially as long as a desired sulfation percentage can be attained, a grade reaction is carried out for 30 minutes - 20 days at 40-90 degrees C, for example.
[0019] The purification actuation regularly used by various qualification polysaccharide can refine the multi-sulfation mucopolysaccharide generated as mentioned above. For example, actuation of collecting neutralization, demineralization by dialysis, and precipitate by organic solvent addition, the recovery actuation by freeze drying, etc. are mentioned.
[0020] The inhibitor of this invention and prevention, or a remedy can also include the support which can usually be used and which may be permitted physiologically or pharmacologically, an excipient, an extending agent, a binder, a humid-ized agent, disintegrator, a surfactant, lubricant, a dispersant, a buffer, a preservative, a solubilizing agent, antiseptics, taste masking and an odor-masking agent, or a stabilizer, unless it is not characterized by including a multi-sulfation mucopolysaccharide and the effectiveness of this invention is spoiled.
[0021] Moreover, the inhibitor of this invention and prevention, or a remedy can choose gestalten, such as the various gestalten as physic pharmaceutical preparation, for example, an ointment, plaster, a tablet, lotions, liquids and solutions, suspension, injections, and aerosols, according to the therapy purpose. And a patient's age, the class of illness and extent, and a list can be medicated intramuscular, taking orally, and ****** in a part, membrane, the skin, hypodermically, a vein, and an artery with a pharmaceutical form and an administration format.
[0022] A basis, an emulsifier, a preservative, etc. are mentioned as an additive blended with an ointment. As a basis, higher alcohol, such as fatty acids, such as lows, such as fats and oils, such as hydrocarbons, such as white vaseline and a liquid paraffin, and an soybean, yellow bees wax, and lanolin, stearin acid, and oleic acid, lanolin alcohol, and cetostearyl alcohol, and the ester of those, macro gall, etc. are mentioned. A nonionic surfactant etc. is mentioned as an emulsifier. Timor, methyl parahydroxybenzoate, propyl parahydroxybenzoate, etc. are mentioned as a preservative.
[0023] As an additive blended with plaster thru/or patches, a thickener, a moisturizer, a bulking agent, a cross linking agent, a resolvent, an emulsifier, etc. are mentioned. As a thickener, sodium

alginate, gelatin, methyl cellulose, a carboxyvinyl polymer, sodium polyacrylate, etc. are mentioned. A glycerol and macro gall are mentioned as a moisturizer. A kaolin, a titanium dioxide, a zinc white, etc. are mentioned as a bulking agent. An acetaldehyde, dimethyl ketone, an aluminum sulfate, etc. are mentioned as a cross linking agent. As a resolvent, alcohols, such as ethanol and 2-propanol, and macro gall are mentioned. An anionic detergent, a nonionic surfactant, etc. are mentioned as an emulsifier.
[0024] As an additive blended with injections, pH regulator, a buffer, a stabilizing agent, an isotonizing agent, local anesthetic, etc. are mentioned.
[0025] As an additive in the case of adjusting as an oral agent, an excipient, disintegrator, lubricant, a binder, an odor-masking agent, corrigent, etc. are mentioned.
[0026] The dose of the inhibitor of this invention and prevention, or a remedy is suitably determined as the class of a patient's age, sex, and illness and extent, and a list with a pharmaceutical form and an administration format.
[0027] Although the content of the multi-sulfation mucopolysaccharide in pharmaceutical preparation is suitably determined by the pharmaceutical form, it is about 0.05 - 1 % of the weight much more preferably about 0.001 to 10% of the weight still more preferably about 0.001 to 50% of the weight preferably.
[0028] It sets to this invention and arteriosclerosis etc. is mentioned as a blood vessel illness prevented or treated based on the adhesion inhibitory action of a leucocyte and a blood vessel inner bark.
[0029] It sets to this invention and the skin microbism, graft versus host disease, **********, psoriasis, atopic dermatitis, contact dermatitis, etc. are mentioned as a skin illness prevented or treated based on the adhesion-molecules manifestation inhibitory action of a skin keratinization cell.
[0030]
[Example] Hereafter, although the contents of this invention are concretely explained using an example, this invention is not limited to these.
[0031] Example 1 106 Homo sapiens keratinocytes (the product made from Oriental Yeast: hereafter referred to as KC) were cultivated among 25cm 2 flask of culture of Homo sapiens keratinocyte with the culture medium (it considers as Following KGM) of 5mL(s) which made the serum free medium for KC (the product made from GIBCO; it considers as Following KBM) contain the bovine brain extract (product made from GIBCO) of 50microg/mL, and the human gene recombination epidermal growth factor (the product made from GIBCO; hereafter referred to as EGF) of 5 ng/mL. Pronase processing performed the passage of KC, on 98 well plate, it cultivated and it offered as a sample KC which carried out 2 passages in seeding and the condition of having become subconfluence.
[0032] example 2 ICAM- by the interferon gamma (IFN-gamma) stimulus in KC — by the culture in operation examination 96 well plates, such as MPS which receives actually one shot, the culture supernatant of KC which became subconfluence was removed, and KBM of 200microL/well washed gently. Next, under existence of drugs, such as MPS of the concentration shown in the following table 1, or nonexistence, KGM of 200microL/well which made IFN-gamma of 100 ng/mL contain was added to KC, and was cultivated for 48 hours. Only KGM of 200microL/well was added to the negative control group.
[0033]
[Table 1]

薬 名	濃 度	例 数
陰性対照	-	4ウェル
陽性対照（IFN-γ刺激 50ng/mL）	-	4ウェル
MPS	10μg/mL	4ウェル
MPS	100μg/mL	4ウェル
ヘパリン	100μg/mL	4ウェル
コンドロイチン4-硫酸	100μg/mL	4ウェル
コンドロイチン6-硫酸	100μg/mL	4ウェル

[0034] Supernatant liquid was removed after culture termination and the phosphoric-acid buffer physiological salt solution (hereafter referred to as PBS) of 200microL/well washed KC once. KC — the PLP solution (2% paraformaldehyde —) of 100microL/well A 75mM L-lysine and 10mM Sodium metaperiodate, a 37.5mM phosphoric acid, After fixing by applying pH6.2 and leaving it for 15 minutes at a room temperature, It washed 3 times by PBS of 200microL/well, and blocked by carrying out 100microL/well addition of PBS (it considering as BSA/PBS hereafter) which made BSA (bovine serum albumin) contain 2% further, and leaving it for 20 minutes at a room temperature. Next, 50microL/well addition of 0.2%BSA/PBS which carried out 10microg/mL content of the primary antibody (product made from anti-Homo sapiens ICAM-1 mAb;Upstate Biotechnology) was carried out after removing blocking liquid, and it was left for 30 minutes at the room temperature. Only BSA/PBS was added to the blank well. 50microL/well addition of 0.2%BSA/PBS which carried out 10microg/mL content of the second antibody (product made from anti-mouse IgG(H+L);Vector by which the biotin indicator was carried out) for KC after 3 times washing by PBS of 200microL/well was carried out, and it was left for 30 minutes at the room temperature. 50microL/well addition after 3 times washing and of the ABC-AP (biotin-ized alkaline phosphatase-avidin complex) solution was carried out by PBS of 200microL/well, and it was left for 30 minutes at the room temperature. It washed 3 times by PBS of 200 moremicroL/well, and the pNPP (p-nitrophenyl phosphate) solution (5mM) of 100microL/well was added, and the absorbance in 405nm was measured with the microplate reader after neglect for 10 minutes at the room temperature. A measurement result is shown in drawing 1 and 2.

[0035] The ICAM-1 manifestation of KC which carried out the IFN-gamma stimulus increased notably compared with the group non-taken a measure. MPS controlled the manifestation of ICAM-1 by IFN-gamma intentionally on the concentration dependence target (drawing 1). Moreover, when the ICAM-1 manifestation depressant action of MPS of 100microg/mL which accepted the significant operation was compared with other mucopolysaccharides (heparin, a chondroitin 4-sulfuric acid, chondroitin 6-sulfuric acid) of this concentration, MPS controlled the ICAM-1 manifestation most strongly (drawing 2).

[0036] From these results, MPS has the cell humid depressant action which minded ICAM-1 guided by IFN-gamma at the time of inflammation, and it is suggested that it is still more useful for an inflammation sex skin skin disease.

[0037] In the F-75 flask which carried out the I-beam collagen coat of the culture HUVEC of example 3HUVEC (Homo sapiens umbilical cord intravenous hide cell), FBS (calf embryo blood serum) was cultivated by MCDB131 culture medium containing 10% and ECGF (endothelial cell growth factor; 100 time dilution), and subculture was suitably carried out by EDTA treatment 0.025% trypsin / 0.01%. Seeding was carried out to 24 well plate which carried out the I-beam collagen coat by 4x104 cels / cell density of mL, and the cell was offered as a sample to the trial of adhesion after 24-hour culture. The cell offered as a sample was a passage number 5.

[0038] After having cultivated culture EoL-of EoL-1 cell (eosinophile leucocyte Mr. established cell line) 1 cell by RPMI-1640 culture medium which contains FBS 10%, carrying out subculture suitably by 2x105 to 2x106 cels / cell density of mL (passage number: 13 to 15 or more) and performing the following fluorescence label processing, it offered as a sample to the trial of adhesion.

[0039] After washing EoL-1 cell cultivated as the fluorescence label above of a sample offering cell by PBS, by about 5x106 cels / cell density of mL It suspends in HBSS (Hanks' balancedsalts) containing BCECF-AM (2', 7'-bis(carboxyethyl)-4, or 5-carboxyfluorescein) (5microM) and BSA (0.1%). It incubated in 2/95% air of 5%COs for 30 (37 degrees C) minutes. The cell was washed once by PBS after incubation termination, and it diluted to RPMI-1640 culture medium which contains FBS 1%, and was used for the trial of adhesion.

[0040] HUVEC cultivated on the adhesion test 24 well plate — TNF — MCDB131 culture medium containing alpha (100 U/mL) and FBS (10%) was added, and it incubated for 4 hours. HUVEC was washed once by PBS after incubation termination, and 4x105 fluorescence labeling EoL-1 cells per two were added 1cm with MPS (0.1% and 1%). It incubated for 30 minutes, carrying out rotation shaking (150rpm) horizontally on (37 degrees C) and a plate shaker in 2/95% air of 5%COs. It removed after incubation termination by washing once EoL-1 cell which has not

been pasted up on HUVEC by PBS. EoL-1 cell pasted up on HUVEC was dissolved by NaOH (1M), and extant fluorescence intensity was measured (excitation; 485nm, fluorescence;530nm). A measurement result is shown in drawing 3 .

[0041] As for MPS, it turns out that the adhesion reaction of EoL-1 cell and HUVEC is controlled and selectin dependency cell adhesion inhibitory action is shown so that more clearly than drawing 3 .

[0042]

[Effect of the Invention] The multi-sulfation mucopolysaccharide is useful as prevention of the skin microbism etc., or a remedy as a selectin dependency cell adhesion inhibitor, the adhesion-molecules manifestation inhibitor of a skin keratinization cell, blood vessel intima hyperplasy, and a vasoconstriction inhibitor by selectin dependency cell adhesion inhibitory action, the adhesion-molecules manifestation inhibitory action of a skin keratinization cell, etc.

[0043] It is useful especially as prevention or the remedy of the blood vessel illness based on selectin dependency cell adhesion inhibitory action, and especially useful as prevention or the remedy of arteriosclerosis.

[0044] Furthermore, it is useful as prevention or the remedy of skin disease, such as the psoriasis and the skin microbism based on the adhesion-molecules manifestation inhibitory action of a skin keratinization cell, graft versus host disease, and **********.

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DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] ICAM- of KC which carried out the IFN-gamma stimulus -- it is the graph which shows the effect of MPS (0.1% and 1%) which receives actually one shot.
[Drawing 2] ICAM- of KC which carried out the IFN-gamma stimulus -- it is the graph which shows the effect of various mucopolysaccharides which receives actually one shot.
[Drawing 3] It is the graph which shows the effect of MPS to adhesion with EoL-1 cell and HUVEC.

[Translation done.]